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DATA EVALUATION RECORD

STUDY TYPE: Subchronic Neurotoxicity – Oral (gavage) Study in Rats, OPPTS 870.6200; (OECD 424).

PC CODE: 088001 **DP BARCODE:** D339760

TEST MATERIAL (PURITY): Copper Omadine (96.8% a.i.)

SYNONYMS: Copper pyrithione; Copper 2-pyridine, CuPT

CITATION: Barnett, J. (2006). Oral (gavage) Subchronic Neurotoxicity Study of Copper Omadine (CuPT) in Rats. Charles River Laboratories, Preclinical Services, Horsham, PA. Laboratory Project ID: AEN00008, December 29, 2006. MRID 47023701. Unpublished.

SPONSOR: Arch Chemicals, Inc., 350 Knotter Drive, Cheshire, CT 06410-0586

EXECUTIVE SUMMARY - In a subchronic neurotoxicity study (MRID 47023701), copper omadine (96.8% a.i., Lot No. 0103239911) in aqueous 0.5% carboxymethylcellulose was administered orally via gavage (10 mL/kg) once daily to 16 Sprague-Dawley rats/sex/dose at doses of 0 and 2.25 mg/kg/day and to 10 rats/sex/dose at 0.5 and 1.25 mg/kg/day for 91 consecutive days. At the end of the dosing period, a subset of 10 rats/sex/dose from the control and 2.25 mg/kg/day groups were allowed to recover for an additional 6 weeks. Neurobehavioral assessment (functional observational battery [FOB] and motor activity testing) was performed on all animals at pre-dosing and Weeks 2, 4, 8, and 13. At study termination, 5 rats/sex/group were anesthetized and perfused *in situ* for neuropathological examination. The tissues from the perfused animals in the control and 2.25 mg/kg/day groups were subjected to histopathological evaluation of brain and peripheral nervous system tissues. Acceptable positive control data were provided.

No compound-related effects were observed in clinical signs of toxicity, body weight, body weight gain, food consumption, ophthalmoscopic effects, FOB, motor activity, brain weights, or gross pathology.

At 2.25 mg/kg/day, the incidence of excessive salivation was increased during the dosage period in both sexes. One female (#16197) was sacrificed *in extremis* on Day 89, and prior to sacrifice, this animal displayed adverse clinical signs that correlated with those previously observed with this test material in the range finding study. An increased ($p \leq 0.01$) incidence of slightly reduced

hindlimb muscle mass was noted at Weeks 6 through 13 in the males and during Weeks 4 through 13 in the females. During the recovery period, males showed signs of recovery; however, the majority of the females still displayed decreased ($p \leq 0.01$) muscle mass up to Week 19. The females also had a corresponding decrease ($p \leq 0.01$) in the average maximum amplitude in the electrophysiological measurements of Compound Motor Action Potential (CMAP) (decr 28%) that remained reduced, but to a lesser extent (decr 13%), following the 6-week recovery period. Treatment-related neuropathological were limited to the skeletal muscle sections in the 2.25 mg/kg/day animals, with definitive muscle fiber atrophy in 1/5 (minimal) males and 3/4 (mild to moderate) females. Additional findings in the muscle of these animals included minimal to mild myositis, mild muscle fiber degeneration, and minimal muscle fiber necrosis.

At 1.25 mg/kg/day, treatment-related effects were limited to an increased incidence of excessive salivation during the dosage period in 4/10 males and 3/10 females. This not-toxicological effects may have been caused by irritation of the oral mucosa.

The LOAEL is 2.25 mg/kg/day based on mortality, reduced hindlimb muscle mass, decreased electrophysiological measurements (females), and microscopic lesions in the skeletal muscle. The NOAEL is 1.25 mg/kg/day.

The study is classified as **Acceptable/Guideline** and satisfies the guideline requirement (OPPTS 870.6200) for a subchronic neurotoxicity study in rats.

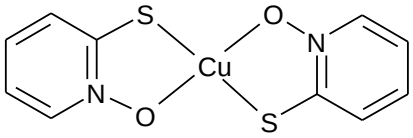
COMPLIANCE - Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Copper omadine

Description: Green powder
Lot #: 0103239911
Purity: 96.8% a.i.
Stability: The test material was shown to be stable in the vehicle for up to 12 days at 5±3°C.
CAS # of TGAI: 14915-37-8
Structure:



2. Vehicle – Aqueous 5% carboxymethylcellulose

3. Test animals

Species:	Rat	
Strain:	Crl:CD(SD)	
Age/weight at dosing:	Approximately 6-7 weeks / 144-170 g males; 137-171 g females	
Source:	Charles River Laboratories (Kingston, NY)	
Housing:	Individually in stainless steel wire-mesh cages	
Diet:	Rodent Lab Chow #5002 (PMI Nutrition International, St. Louis MO), *ad libitum*, except during neurobehavioral testing	
Water:	Reverse osmosis treated tap water (with chlorine added as a bacteriostat), *ad libitum*, except during neurobehavioral testing	
Environmental conditions:	**Temperature:**	19-25°C
	Humidity:	30-70%
	Air changes:	≥10/hr
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	At least 5 days	

B. STUDY DESIGN

1. In-life dates - Start: 04/25/06 End: 07/27/06

2. Animal assignment and dose rationale – The animals were randomly assigned (stratified by body weight) to the test groups noted in Table 1. Subsets of 10 rats/sex/dose in the control and 2.25 mg/kg/day groups were allowed to recover for approximately 6 weeks after the end of the dosing period. The doses for the current study were selected based on the results of a previously conducted 28-day range-finding study (CRL Study No. AEN00007). This study is summarized and included as an Appendix to this DER.

TABLE 1. Study design [a]				
Experimental parameter	**Dose (mg/kg/day)**			
	0	**0.5**	**1.25**	**2.25**
Total number of animals/sex/group	16/sex	10/sex	10/sex	16/sex
Behavioral testing (FOB, Motor activity)	16/sex	10/sex	10/sex	16/sex
Electrophysiological testing	16/sex	10/sex	10/sex	16/sex
Neuropathology	5/sex	5/sex	5/sex	5/sex
Recovery group	10/sex	-	-	10/sex

[a] Data were extracted from pages 27, 30, & 31 of the study report.

3. **Test substance preparation, administration, and analysis** – Test formulations were prepared daily by mixing the appropriate amount of the test material with aqueous 5% carboxymethylcellulose. Formulations were stirred continuously during dosing. Rats were dosed once daily via oral gavage for 91 consecutive days. Dose volume was adjusted daily based on individual body weights. Homogeneity (top, middle, and bottom) was determined from samples collected at the start and end of the study. In a range-finding study (CRL Study No. AEN00007), test formulations were shown to be stable for up to 12 days at 5±3°C at concentrations of 0.25 and 2.50 mg/kg/day (which bracketed the range in the current study). Actual concentration at each dose was verified at Weeks 1, 2, 4, 8, 9, and 13 in the current study.

Results

Homogeneity analysis (range as % relative standard deviation, RSD): 1.5-7.9%

Concentration analysis (range as mean % of nominal):

Concentration (mg/mL)	% of nominal
0.05	95.0-104.6
0.125	92.4-101.6
0.225	85.6-114.4

The actual concentration of the test material was outside the acceptable range in the 0.225 mg/mL formulation at Week 8 (85.6% of nominal). Analysis of backup samples could not confirm that the original results were outside the acceptable range (108.2% of nominal). Otherwise, the variation between nominal and actual dosage was acceptable. The homogeneity of both (original and backup) of the 0.225 mg/mL samples at Week 1 were outside the acceptable range of ≤5% RSD (7.9 and 5.8% RSD, respectively). Otherwise, the analytical data indicated that the mixing procedure was adequate at all other concentrations at the beginning and end of the treatment period.

4. Statistics – The following statistical methods were applied to the data:

Parameter	Statistical Procedures
FOB parameters using interval scales (such as grip strength tests and landing foot splay test), body weight data, food consumption	Bartlett's test was performed. If the result was not significant ($p>0.001$), then ANOVA was performed. When ANOVA was significant ($p\leq 0.05$), Dunnett's test was conducted. If Bartlett's test revealed heterogeneity of variances, the Kruskal-Wallis test was performed when 75% or fewer of the scores in all the groups were tied, and when this test result was significant ($p\leq 0.05$), Dunn's test was conducted. When more than 75% of the scores in any group were tied, Fisher's Exact Test was used to compare the proportion of ties in the groups.
Motor activity	Analysis of Variance with Repeated Measures was performed. Significance ($p\leq 0.05$) was tested based on a difference between groups in the total across all measurements in a session (effect of Concentration) and on a difference between groups at specific measurement periods (interaction between Concentration and Block). If the Concentration effect was significant, Dunnett's test was performed. If the Concentration x Block interaction was significant, ANOVA test was used to evaluate the data at each measurement period, and a significant result ($p\leq 0.05$) was followed by a Dunnett's test.
FOB having graded or count scores	The Kruskal-Wallis test was performed when 75% or fewer of the scores in all the groups were tied, and when this test result was significant ($p\leq 0.05$), Dunn's test was conducted. When more than 75% of the scores in any group were tied, Fisher's Exact Test was used to compare the proportion of ties in the groups.
Clinical observation incidence data, FOB descriptive and quantal data	Contingency tables using the Variance Test for Homogeneity of the Binomial Distribution were used.
Neuropathology findings	The Fisher's Exact Test was conducted.

Statistical significance was denoted as either $p\leq 0.05$ or $p\leq 0.01$. The reviewers consider the analyses used to be appropriate.

C. METHODS / OBSERVATIONS

1. Mortality and clinical observations - Animals were observed twice daily (prior to daily dosing and at 60±10 minutes post-dosing) for mortality and clinical signs of toxicity. Detailed clinical observations were performed weekly throughout the pre-dosing, dosing, and post-dosing (recovery) periods. Additionally, muscle mass was evaluated in all rats once per week (prior to daily dosing). The technician "rolled" the calf muscles between his or her thumb and forefinger and evaluated the muscle mass subjectively by digital palpation.

2. Body weight - Animals were weighed on the days that the FOB was performed (pre-exposure and Weeks 2, 4, 8, and 13), daily during the exposure period, weekly during the recovery period, and at termination.

3. Food consumption – Food consumption was recorded weekly during pre-dosing, dosing, and post-dosing (recovery), and the mean absolute (g/animal/day) and relative (to body weight; g/kg/day) values were reported.

4. Cholinesterase determination - Cholinesterase activity was not determined.

5. Ophthalmoscopic examinations – The eyes of all animals were examined pre-dosing and just prior to termination.

6. Neurobehavioral assessment

a. Functional Observational Battery (FOB) – The FOB was conducted prior to initiation of dosing and at Weeks 2, 4, 8, and 13. The technician conducting the evaluations was unaware of the dose group assignment of the animals. The open field evaluation was approximately 2 minutes in duration. The scoring criteria were not provided. The following CHECKED (X) parameters were examined.

	HOME CAGE OBSERVATIONS		HANDLING OBSERVATIONS		OPEN FIELD OBSERVATIONS
X	Posture*	X	Reactivity*		Mobility
	Biting	X	Lacrimation* / chromodacryorrhea	X	Rearing±
X	Convulsions*	X	Salivation*	X	Arousal/ general activity level*
X	Tremors*	X	Piloerection*	X	Convulsions*
X	Abnormal Movements*	X	Fur appearance	X	Tremors*
	Palpebral closure*	X	Palpebral closure*	X	Abnormal movements*
	Feces consistency	X	Respiratory rate±	X	Urination / defecation*
		X	Red/crusty deposits*	X	Grooming
	SENSORY OBSERVATIONS	X	Mucous membranes /eye /skin color	X	Gait abnormalities / posture*
X	Approach response±	X	Eye prominence*	X	Gait score*
X	Touch response±	X	Muscle tone*	X	Bizarre / stereotypic behavior*
X	Startle response*	X	Vocalization	X	Backing
X	Pain response*				Time to first step
X	Pupil response*				
	Eye-blink response		**PHYSIOLOGICAL OBSERVATIONS**		**NEUROMUSCULAR OBSERVATIONS**
	Forelimb extension	X	Body weight*		Hindlimb extensor strength
	Hindlimb extension	X	Body temperature±	X	Forelimb grip strength*
X	Air righting reflex±			X	Hindlimb grip strength*
	Olfactory orientation			X	Landing foot splay*
X	Visual placing response		**OTHER OBSERVATIONS**		Rotarod performance

*Required parameters; ±Recommended parameters

b. Locomotor activity - Locomotor activity was evaluated following the FOB (prior to initiation of dosing and at Weeks 2, 4, 8, and 13). The movements of each rat were monitored by a passive infrared sensor mounted outside a stainless steel, wire-bottom cage during a 1.5 hour period, with the number of movements and time spent in movement tabulated at 5-minute intervals. Each rat was tested in the same location on the rack across test sessions, and groups were counterbalanced across test sessions and cages.

c. Electrophysiological testing – Electrophysiological testing was conducted on all male and female rats on the day of final dosing (prior to administration) and on all female rats in the control and 2.25 mg/kg/day recovery groups at the end of the recovery phase of the

study. Maximum compound motor action potential (CMAP) amplitude was recorded from the flexor muscles of the hock after stimulating the sciatic nerve near the hip. The testing was conducted using a Nicolet Viking Quest system (Model #: NG030159) which was calibrated shortly before the first day of testing and shortly after the last day of testing. The technician conducting the studies was unaware of the dose group assignment of the animals. The details of the methodology were reported on pages 31-32 of the study report.

7. **Sacrifice and pathology –** All surviving animals (not assigned to the recovery groups) were sacrificed via exsanguination, after an intravenous injection of a combination of sodium heparin and sodium pentobarbital. The rats were then perfused *in situ* with neutral buffered 10% formalin, and a gross necropsy was performed. Gross lesions were retained in neutral buffered 10% formalin and shipped to Research Pathology Services, Inc. (New Britain, PA) for analysis. The calvaria were removed, and the head was immersed in the fixative. After at least 24 hours, the brains from the animals selected for neurohistological examination (5 rats/sex/dose) were excised and weighed. In rats not selected for neurohistological examination, the vertebral column was cut into segments, and the hindlimbs were removed and dissected to expose the peripheral nerves. These tissues were immersed in the fixative and, along with the head and brain, retained for possible evaluation.

   In the animals selected for evaluation of neuropathological effects (5 rats/sex/dose), tissues were further dissected to allow the evaluation of the Gasserian ganglion, spinal cord, peripheral nerves, skeletal muscles, brain, and eye as detailed in the table below. The following CHECKED (X) tissues from the control and 2.25 mg/kg/day groups were evaluated microscopically by CRL Pathology Associates (Frederick, MD).

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Forebrain		Mid-thigh
X	Center of cerebrum	X	Sciatic notch
X	Midbrain		
X	Cerebellum		**OTHER**
X	Pons	X	Sural nerve
X	Medulla oblongata	X	Tibial nerve (proximal and distal)
	SPINAL CORD	X	Peroneal nerve (fibular)
X	Cervical swelling	X	Lumbar dorsal root ganglion
X	Thoracic swelling	X	Lumbar dorsal root fibers
X	Lumbar swelling	X	Lumbar ventral root fibers
	OTHER	X	Cervical dorsal root ganglion
X	Gasserian ganglion	X	Cervical dorsal root fibers
X	Trigeminal nerves	X	Cervical ventral root fibers
X	Optic nerve	X	Thoracic dorsal root ganglion
X	Eyes	X	Thoracic dorsal root fibers
X	Gastrocnemius muscle	X	Thoracic ventral root fibers
	Anterior tibial muscle		

   The brain, spinal cord, and eyes (with optic nerves and retina) were trimmed, processed, embedded in paraffin, and sectioned 3 times. Each section was stained with hematoxylin and

eosin (H&E), luxol fast blue/cresyl violet, and Bielschowsky's. The skeletal muscle was trimmed, processed, embedded in paraffin, sectioned, and stained with H&E. The nerves (including the Gasserian ganglia) were embedded in plastic, and sectioned 3 times at approximately 2 µm each. Each section was stained with H&E, toluidine blue, and Bielschowsky's. Longitudinal and transverse sections were made of the cervical, thoracic, and lumbar spinal cord; and the sciatic, tibial, fibular, and sural nerves.

8. **Positive controls** - Summary data from two studies (Argus Study Nos. 012-058 and 012-104; performed in 1996 and 2002, respectively) were provided that generated positive control data and validated the procedures and observers of the performing lab to conduct the FOB and to assess motor activity effects. In the FOB portion of this study, exposure to iminodipropionitrile (**IDPN**; 250 mg/kg; 4/5 daily i.p. doses at 1 mL/kg) induced the following effects at 3 days post-dosing: (i) stereotypic behavior; (ii) slight ataxia; (iii) increased ($p<0.05$ gait abnormality; (iv) impaired air-righting reflex; (v) decreased ($p<0.05$) hindlimb grip strength; and (vi) decreased ($p<0.01$) body weight in the males. At 10 days post-dosing, additional signs observed included increased ($p<0.01$) foot splay in the males and abnormal respiratory rate in both sexes. The following effects were noted at 60 minutes post-dosing with **chlorpromazine** (6 mg/kg; single i.p. dose at 1 mL/kg): (i) unusual posture; (ii) decreased mobility in the open-field; (iii) palpebral closure (eyes half-closed); (iv) lacrimation; (v) impaired air-righting reflex; and (vi) decreased ($p<0.01$) body temperature in the males. Exposure to **d-amphetamine** (4 mg/kg; single i.p. dose at 1 mL/kg) induced stereotypic behavior and piloerection at 30 minutes post-dosing. **Carbaryl** (100 mg/kg; single oral dose at 4 mL/kg) induced the following effects at 30 minutes post-dosing: (i) unusual behavior; (ii) whole body tremors; (iii) limb twitches; (iv) unusual posture; (v) slight to moderately impaired gait (ataxic, limbs splayed/dragging, or tip-toe); (vi) lacrimation; (vii) salivation; (viii) decreased pupil response; (ix) decreased ($p<0.01$) body temperature; and (x) increased ($p<0.05$) foot splay in females. Exposure to dichlorodiphenyltrichloroethane (**DDT;** 75 mg/kg; single oral dose at 2 mL/kg) induced the following effects at 6 hours post-dosing: (i) unusual behavior; (ii) limb twitches; (iii) whole body tremors; (iv) abnormal respiration; and (v) impaired air-righting reflex. In the motor activity test, exposure to **acrylamide** (45 mg/kg; 10 daily i.p. doses at 1 mL/kg) induced decreases ($p<0.05$) in the number of moves and time spent in motion in both sexes, and **d-amphetamine** (0.75 mg/kg; 3 i.p. doses at 1 mL/kg) induced increases ($p<0.05$) in the number of moves and time spent in motion in both sexes. No positive control data that demonstrate the ability of the performing laboratory to identify neuropathological lesions were provided. However, as the tissue samples were sent to an independent pathology laboratory for evaluation, this lack of data is not considered to be a deficiency.

II. RESULTS

A. OBSERVATIONS

1. **Clinical signs –** With the exception of the signs observed in the 2.25 mg/kg/day female that was sacrificed on Day 89 (see below), no adverse treatment-related clinical signs were observed at any dose in either sex. An increased incidence of excessive salivation was noted during the dosage period in the males and females at ≥1.25 mg/kg/day. It was stated that copper omadine has been shown to be a severe irritant to the mucosal membranes and an increase in salivation following oral administration would be expected.

2. **Mortality –** At 2.25 mg/kg/day, one female (#16197) was sacrificed *in extremis* on Day 89. This animal displayed the following adverse clinical signs prior to sacrifice: (i) sparse hair coat on both forelimbs; (ii) localized alopecia on the limbs; (iii) chromodacryorrhea; (iv) ataxia; (v) limited use of both hindlimbs; (vi) hunched posture; (vii) dehydration; (vii) chromorhinorrhea; (ix) red perinasal substance; (x) emaciation; and (xi) pale extremities. Additionally, this animal displayed decreased body weight and feed consumption. FOB effects observed included decreased arousal, hunched posture, limited use of both hindlimbs, dehydration, alopecia, impaired surface righting, reduced fore- and hindlimb strength, and decreased motor activity during Week 13. Also, this animal displayed slightly reduced muscle mass from Week 4 until Week 12 when it was observed to be greatly reduced. No treatment-related gross or histopathological findings were noted. It was stated that this death was considered to be related to treatment because the clinical signs observed correlate with those previously observed with this test material. One 1.25 mg/kg/day male (#16101) was found dead on Day 48. This death was considered incidental and unrelated to treatment. All other animals survived to scheduled sacrifice.

3. **Muscle mass evaluation –** At 2.25 mg/kg/day, an increased ($p \leq 0.01$) incidence (# affected/16 vs. 0 controls) of slightly reduced hindlimb muscle mass was noted at Weeks 6 through 13 in the males and during Weeks 4 through 13 in the females (Table 2a). During the recovery period, males showed signs of recovery; however, the majority of the females still displayed decreased ($p \leq 0.01$) muscle mass up to Week 19 (Table 2b).

TABLE 2a. Incidence (# affected) of decreased muscle mass in rats treated with copper omadine via gavage for 91 days. [a]

Week	Severity	Dose (mg/kg/day)							
		0		0.5		1.25		2.25	
		Male	Female	Male	Female	Male	Female	Male	Female
1	Slightly reduced	0	0	0	0	0	0	0	0
	Normal	16	16	10	10	10	10	16	16
4	Slightly reduced	0	0	0	0	0	0	0	15**
	Normal	16	16	10	10	10	10	16	1*
5	Slightly reduced	0	0	0	0	0	0	3	16**
	Normal	16	16	10	10	10	10	13	0*
6	Slightly reduced	0	0	0	0	0	0	4**	16**
	Normal	16	16	10	10	10	10	12**	0*
9	Slightly reduced	0	0	0	0	0	0	9**	16**
	Normal	16	16	10	10	9[b]	10	7**	0*
11	Slightly reduced	0	0	0	0	0	0	12**	15**
	Normal	16	16	10	10	9	10	4**	1*
12	Greatly reduced	0	0	0	0	0	0	0	1
	Slightly reduced	0	0	0	0	0	0	5**	14**
	Normal	16	16	10	10	9	10	11**	1*
13	Greatly reduced	0	0	0	1	0	0	0	0
	Slightly reduced	0	0	0	0	0	0	6**	15**[c]
	Normal	16	16	10	9	9	10	10**	0**

[a] Data were extracted from Tables 7 and 8 on pages 136-145 of the study report; n=10 for the 0.5 and 1.25 mg/kg/day groups and n=16 for the control and 2.25 mg/kg/day groups.

[b] Excludes values for animal # 16101, which was found dead on Day 48.

[c] Excludes values for animal # 16197, which was sacrificed on Day 89 due to adverse clinical observations.

** Statistically significantly different from controls at $p \leq 0.01$

TABLE 2b. Incidence (# affected) of decreased muscle mass in rats treated with copper omadine via gavage for 91 days and allowed to recover for an additional 6 weeks. [a]

Week	Severity	Dose (mg/kg/day)							
		0		0.5		1.25		2.25	
		Male	Female	Male	Female	Male	Female	Male	Female [b]
14	Slightly reduced	0	0	-	-	-	-	3	9**
	Normal	10	10	-	-	-	-	7	0**
15	Slightly reduced	0	0	-	-	-	-	1	9**
	Normal	10	10	-	-	-	-	9	0**
16	Slightly reduced	0	0	-	-	-	-	1	8**
	Normal	10	10	-	-	-	-	9	1**
17	Slightly reduced	0	0	-	-	-	-	0	9**
	Normal	10	10	-	-	-	-	10	0**
19	Slightly reduced	0	0	-	-	-	-	0	7**
	Normal	10	10	-	-	-	-	10	2**

a Data were extracted from Tables 7 and 8 on pages 139-140 and 144-145 of the study report; n=10.

b Excludes values for animal # 16197, which was sacrificed on Day 89 due to adverse clinical observations.

****** Statistically significantly different from controls at $p \leq 0.01$

B. <u>BODY WEIGHT AND BODY WEIGHT GAIN</u> – No adverse effects on body weight or body weight gain were noted at any dose in either sex during the dosing or recovery periods (Table 3). All statistically significant differences noted were considered incidental because they were either not adverse, not dose-dependent, and/or were transient.

TABLE 3. Mean (±SD) body weight and overall (Days 1-91) body weight gain (g) in rats treated with copper omadine via gavage for up to 91 days. [a]

Days	Dose (mg/kg/day)			
	0	0.5	1.25	2.25
Males				
1	185.2±14.4	185.5±16.0	184.4±13.4	184.1±15.2
43	441.0±35.1	431.8±34.7	439.4±36.7	429.1±45.6
91	554.9±53.6	548.2±54.9	559.4±36.7	541.2±56.3
Overall (Days 1-91) gain	369.7±50.2	362.7±55.0	375.4±34.9	357.1±47.2
99	568.7±62.3	-	-	590.4±42.5
134	619.0±66.1	-	-	650.5±36.3
Overall (Days 91-134) gain	87.3±13.4	-	-	99.5±13.3
Overall (Days 1-134) gain	444.6±60.8	-	-	481.7±33.4
Females				
1	168.3±12.0	169.4±14.0	168.4±11.8	169.4±12.9
43	274.9±25.1	272.9±11.6	282.5±17.6	269.8±26.4
91	309.4±32.5	305.3±26.2	323.2±22.1	305.3±32.8
Overall (Days 1-91) gain	141.1±29.4	135.9±22.8	154.8±22.0	136.4±24.9
99	315.9±32.3	-	-	309.9±32.0
134	333.1±35.1	-	-	337.2±40.3
Overall (Days 91-134) gain	30.3±11.9	-	-	38.7±13.9
Overall (Days 1-134) gain	169.0±35.8	-	-	178.6±32.8

[a] Data were extracted from Tables 11-14 on pages 166-173 of the study report; n=10 for the 0.5 and 1.25 mg/kg/day groups and n=16 for the control and 2.25 mg/kg/day groups.

C. FOOD CONSUMPTION – No adverse treatment-related effects were observed on absolute or relative food consumption in either sex at any dose. The statistically significant differences noted were not considered biologically important because they were increased rather than decreased, they were minor (<12%), and the increases in absolute food consumption did not correspond with increases in relative food consumption.

D. OPHTHALMOSCOPIC EXAMINATIONS – No treatment-related ocular lesions were observed at any dose at the end of the dosage period.

E. NEUROBEHAVIORAL RESULTS

1. FOB findings – No treatment-related FOB effects were noted at any dose in either sex. All statistically significant findings were considered incidental because they were either (i) considered normal behavior for a given parameter, (ii) did not correspond with any unusual values in any other FOB parameter, (iii) not comparable between sexes, or (iv) not dose-dependent.

2. Electrophysiological measurements – In the 2.25 mg/kg/day females, the maximum amplitude of the CMAP was decreased ($p \leq 0.01$) by 28% compared to controls at the end of the dosing period (Day 91; Table 4). At the end of the recovery period (Day 134), the maximum amplitude was still decreased ($p \leq 0.05$) by 13% in the 2.25 mg/kg/day females compared to controls; however, the reduction was smaller compared to the end of the dosing period, which indicates that some recovery had occurred.

TABLE 4. Mean (±SD) electrophysiological measurements of compound motor action potential (maximum amplitude, mV) in rats treated with copper omadine via gavage for 91 days and allowed to recover for an additional 6 Weeks. [a]

Observation	Dose (mg/kg/day)			
	0	0.5	1.25	2.25
Males (Day 91)	51.9±11.5	48.4±13.6	47.1±10.8	49.6±9.6
Females (Day 91)	51.6±8.9	51.9±7.8	52.2±12.0	37.1±15.6**(↓28)
Females (Day 134)	63.0±9.4	-	-	54.9±5.8*(↓13)

a Data were extracted from Tables 19 and 20 on pages 182-183 of the study report; n=10 for the 0.5 and 1.25 mg/kg/day groups and n=16 for the control and 2.25 mg/kg/day groups on Day 91, n=10 in the controls and n=9 in the 2.25 mg/kg/day group on Day 134. Percent difference from controls (calculated by reviewers) is presented parenthetically.

***** Statistically significantly different from controls at $p \leq 0.05$

****** Statistically significantly different from controls at $p \leq 0.01$

3. **Motor activity –** No treatment-related effects on total session motor activity (number of movements or time spent in movement) were observed at any dose in either sex (Table 5). Interval motor activity data were provided in Tables 9 and 10 on pages 146-165. Habituation was unaffected by treatment.

TABLE 5. Mean (±SD) total session motor activity in rats treated with copper omadine via gavage for up to 91 days. [a]

Observation	Dose (mg/kg/day)			
	0	0.5	1.25	2.25
Males				
Number of movements				
Pretest	496.2±231.0	567.1±154.0	495.9±124.6	539.5±244.1
Week 2	520.2±271.5	632.4±177.2	462.4±151.5	560.7±231.5
Week 4	586.4±199.1	576.6±191.4	615.2±197.1	577.5±202.4
Week 8	575.9±293.6	634.3±231.3	511.6±127.5	740.8±263.5
Week 13	582.5±327.8	537.6±333.8	478.6±222.3	529.8±235.3
Time spent in movement (sec)				
Pretest	725.0±443.5	928.4±199.4	812.9±296.5	835.1±371.4
Week 2	755.4±399.0	1034.6±406.1	696.7±323.0	872.6±396.1
Week 4	851.8±285.1	928.4±376.3	961.2±330.8	888.8±313.7
Week 8	927.1±480.1	1071.8±372.2	963.0±273.0	1267.6±417.8
Week 13	963.2±638.0	878.6±573.2	862.0±460.0	902.2±481.9
Females				
Number of movements				
Pretest	664.8±284.9	717.7±226.3	615.2±185.7	674.4±243.5
Week 2	583.8±200.8	652.9±329.2	588.2±205.3	602.1±185.3
Week 4	690.9±199.6	600.2±201.6	645.9±264.7	709.1±179.8
Week 8	593.9±207.7	659.7±258.3	752.1±316.4	684.0±271.7
Week 13	629.3±237.0	649.7±238.6	614.3±218.2	662.1±324.7
Time spent in movement (sec)				
Pretest	984.2±412.7	1180.8±407.0	897.3±222.3	988.1±386.6
Week 2	803.9±258.6	1030.9±525.9	855.7±323.6	900.6±325.6
Week 4	934.6±308.1	931.9±306.8	1031.3±430.6	1055.5±488.5
Week 8	907.1±378.8	1195.4±453.9	1250.9±569.8	1197.1±634.5
Week 13	989.2±371.8	1100.5±374.1	975.8±523.9	1119.4±706.4

a Data were extracted from Tables 9 and 10 on pages 146-165 of the study report; n=10 for the 0.5 and 1.25 mg/kg/day groups and n=16 for the control and 2.25 mg/kg/day groups.

G. SACRIFICE AND PATHOLOGY

1. **Gross pathology –** No treatment-related gross lesions were noted in any animal.

2. **Brain weights** – No treatment-related differences in absolute or relative (to body) brain weights were observed at any dose in either sex (Table 6). The increase noted in relative (to body) brain weight in the 2.25 mg/kg/day males (↑12%; p≤0.01) was not considered to be related to treatment, but rather due to the decreased (p≤0.01) terminal body weight (↓16%) in the 5 males selected for neuropathological examination.

TABLE 6. Selected mean (±SD) absolute (g) and relative (to body, %) brain weights in rats treated with copper omadine via gavage for 91 days. [a]

Parameter	Dose (mg/kg/day)			
	0	0.5	1.25	2.25
Males				
Terminal Body (g)	572.6±38.2	545.0±27.8	568.2±41.3	483.0±40.1**(↓16)
Absolute	2.316±0.088	2.288±0.099	2.304±0.055	2.196±0.133
Relative (to body)	0.404±0.026	0.418±0.013	0.406±0.023	0.454±0.025**(↑12)
Females				
Terminal Body (g)	316.4±37.3	311.8±13.3	326.0±34.1	288.2±49.9
Absolute	2.095±0.060	2.062±0.118	2.062±0.151	2.105±0.053
Relative (to body)	0.670±0.087	0.662±0.040	0.634±0.050	0.753±0.162

[a] Data were extracted from Tables 23 and 24 on pages 186-187 of the study report; n=5.

3. **Neuropathology** – Treatment-related neuropathological lesions (# affected vs. 0 controls) were limited to the skeletal muscle sections in the 2.25 mg/kg/day animals, with definitive muscle fiber atrophy in 1/5 (minimal) males and 3/4 (mild to moderate) females (Table 7). Additional findings in the muscle of these animals included minimal to mild myositis (2/5 males and 1/4 females), mild muscle fiber degeneration (2/4 females), and minimal muscle fiber necrosis (1/4 females). All other microscopic lesions noted (nerve fiber degeneration and ganglion neuron vacuolation) are commonly observed in this strain and age of rat and/or were observed in a similar or greater frequency in the controls.

TABLE 7. Neuropathological lesions in the skeletal muscle of rats treated with copper omadine via gavage for 91 days. [a]

Observation	Severity	Dose (mg/kg/day)			
		0	2.25	0	2.25
		Males		Females	
Muscle fiber atrophy	Minimal	0	1	0	0
	Mild	0	0	0	1
	Moderate	0	0	0	2
	Total	0	1	0	3*
Myositis	Minimal	0	1	0	0
	Mild	0	1	0	1
	Total	0	2	0	1
Muscle fiber degeneration	Mild	0	0	0	2
Muscle fiber necrosis	Minimal	0	0	0	1

[a] Data were extracted from Tables 1 and 2 of the pathology report on pages 988 and 994 of the study report; n=5, except for n=4 in the 2.25 mg/kg/day females.

* Statistically significantly different from controls at p≤0.05

III. DISCUSSION AND CONCLUSIONS

A. <u>CONCLUSIONS</u> – No compound-related effects were observed in clinical signs of toxicity, body weight, body weight gain, food consumption, ophthalmoscopic effects, FOB, motor activity, brain weights, or gross pathology.

At 2.25 mg/kg/day, the incidence of excessive salivation was increased during the dosage period in both sexes. It was stated that this effect was presumed to be due to the irritation of the oral mucosa. One female (#16197) was sacrificed *in extremis* on Day 89, and prior to sacrifice, this animal displayed adverse clinical signs that correlated with those previously observed with this test material. An increased ($p \leq 0.01$) incidence (# affected/16 vs. 0 controls) of slightly reduced hindlimb muscle mass was noted at Weeks 6 through 13 in the males (4-12) and during Weeks 4 through 13 in the females (15-16). During the recovery period, males showed signs of recovery; however, the majority of the females still displayed decreased ($p \leq 0.01$) muscle mass up to Week 19. The females also had a corresponding decrease ($p \leq 0.01$) in the average maximum amplitude in the electrophysiological measurements of CMAP (↓28%) that remained reduced, but to a lesser extent (↓13%), following the 6-week recovery period. Treatment-related neuropathological lesions (# affected vs. 0 controls) were limited to the skeletal muscle sections in the 2.25 mg/kg/day animals, with definitive muscle fiber atrophy in 1/5 (minimal) males and 3/4 (mild to moderate) females. Additional findings in the muscle of these animals included minimal to mild myositis, mild muscle fiber degeneration, and minimal muscle fiber necrosis.

At 1.25 mg/kg/day, treatment-related effects were limited to an increased incidence of excessive salivation during the dosage period in the males and females. The not-toxicological significant effects may have been due to the irritation of the oral mucosa.

The LOAEL is 2.25 mg/kg/day based on mortality, reduced hindlimb muscle mass, decreased electrophysiological measurements (females), and microscopic lesions in the skeletal muscle. The NOAEL is 1.25 mg/kg/day.

The study is classified as **Acceptable/Guideline** and satisfies the guideline requirement (OPPTS 870.6200) for a subchronic neurotoxicity study in rats.

B. <u>STUDY DEFICIENCIES</u> – None

APPENDIX

28-Day Dose Range-finding Study in Rats

Since this is a range-finding study, only a summary is provided to confirm the adequacy of the dose selection rationale used in the definitive subchronic oral neurotoxicity study in rats (MRID 47023701).

In a 28-day oral toxicity study (Charles River Study No. AEN00007), copper omadine (96.8% a.i., Lot No. 0103239911) in aqueous 0.5% carboxymethylcellulose was initially administered orally via gavage (10 mL/kg) once daily to 5 Sprague-Dawley rats/sex/dose at doses of 0, 0.25, 0.5, 1.25, or 2.50 mg/kg/day for 28 consecutive days. The females assigned to the 2.50 mg/kg/day group received that dose from Days 1-17; however, due to the toxicity observed, treatment was discontinued for 5 days until an improvement in their condition was observed and the dosage was reduced to 1.75 mg/kg/day. The lower dosage was administered for 14 additional days (Days 22-35).

At 1.25 mg/kg/day, reduced body weight gains were observed in both sexes.

At 2.50 mg/kg/day, the following treatment-related effects were observed: (i) adverse clinical signs (hunched posture, shuffling gait, scant feces, limited use of hindlimbs, dehydration, ungroomed coat, chromodacryorrhea, and pale extremities); (ii) mortality (sacrifice of one female rat, due to the severity of clinical signs mentioned above); (iii) decreased body weight and body weight gain; (iv) decreased food consumption; (v) reduced hindlimb muscle mass; and (vi) reduced grip strength.

The LOAEL is 1.25 mg/kg/day based upon decreased body weight gains in both sexes. The NOAEL is 0.50 mg/kg/day.

This study is classified as **Acceptable/Non-guideline.**

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

Sign-off Date : 02/15/11
DP Barcode Nos.: D375749 and D369393

TXR Nos. : 1,003,204